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Picophytoplankton responses to changing nutrient and light regimes during a bloom

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Abstract The spring bloom in seasonally stratified seas is often characterized by a rapid increase in photosynthetic biomass. To clarify how the combined effects of nutrient and light availability influence phytoplankton composition in the oligotrophic Gulf of Aqaba, Red Sea, phytoplankton growth and acclimation responses to various nutrient and light regimes were recorded in three independent bioassays and during a naturally-occurring bloom. We show that picoeukaryotes and *Synechococcus* maintained a "bloomer" growth strategy, which allowed them to grow quickly when nutrient and light limitation were reversed. During the bloom picoeukaryotes and *Synechococcus* appeared to have higher P requirements relative to N, and were responsible for the majority of photosynthetic biomass accumulation. Following stratification events, populations limited by light showed rapid photoacclimation (based on analysis of cellular fluorescence levels and photosystem II photosynthetic efficiency) and community composition shifts without substantial changes in photosynthetic biomass. The traditional interpretation of "bloom" dynamics (i.e., as an increase in photosynthetic biomass) may therefore be confined to the upper euphotic zone where light is not limiting, while other acclimation processes are more ecologically relevant at depth. Characterizing acclimation processes and growth strategies is important if we are to clarify mechanisms that underlie productivity in oligotrophic regions, which account for approximately half of the global primary production in the ocean. This information is also important for predicting how phytoplankton may respond to global warming-induced oligotrophic ocean expansion.

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Introduction

Phytoplankton blooms occur when the rates of cell growth and division significantly exceed the rates of cell loss, resulting in rapid biomass accumulation. Sufficient nutrients and light, as well as an absence of grazers, are conditions that stimulate phytoplankton growth. A transient occurrence of any of these favorable growth factors can be the causal event in bloom initiation. For example, mixing periods in permanently and seasonally stratified oligotrophic seas can result in episodic inputs of growth-limiting nutrients that trigger rapid, transient increases in photosynthetic biomass (i.e., blooms). Blooms are also initiated following delivery of exogenous nutrients via processes such as nitrogen fixation and atmospheric deposition. The nature and magnitude of a bloom is linked to composition of the phytoplankton community, which determines growth rates, acclimation strategies, and competitive interactions that ensue during the bloom. However, understanding how

nutrient levels and physiological factors contribute to bloom dynamics is confounded by the ambient physical characteristics of the water column, where light may supersede nutrient availability as the growth limiting variable.

The Gulf of Aqaba, an oligotrophic water body fed by nutrient-depleted surface waters from the Red Sea, has seasonal cycles of stratification and mixing similar to other subtropical oligotrophic seas. Its water column is stratified during summer, and surface water nutrient levels are near the limits of detection (Levanon-Spanier et al. 1979; Reiss and Hottinger 1984; Mackey et al. 2007). During the summer months atmospheric dry deposition is a significant source of nutrients to the euphotic zone, supporting transient phytoplankton blooms (Chen et al. 2007; Paytan et al. 2009). Beginning in the fall, cooling of surface waters initiates convective mixing, and a deeply mixed (300 m or more) water body is observed by winter (Wolf-Vetch et al. 1992). Nutrients entrained from depth as a consequence of this mixing result in mesotrophic conditions (Lindell and Post 1995), with productivity likely limited by light (Labiosa et al. 2003). The water column begins to re-stratify in the spring as surface waters warm, trapping nutrients and phytoplankton in the euphotic zone along a steep light gradient. Phytoplankton take advantage of the favorable nutrient and light conditions within the newly stratified euphotic zone forming a surface bloom over a period of days (Labiosa 2007), while the growth of phytoplankton trapped at depth is limited by low light. Picophytoplankton (cells < 2 μm) are the dominant cell type in the Gulf of Aqaba; however, ultraplankton (cells < 8 μm) and some larger diatoms and dinoflagellates (cells 5–100 μm) also occur in phytoplankton assemblages, particularly during the mesotrophic winter season (Lindell and Post 1995; Sommer 2000; Mackey et al. 2007).

Permanently and seasonally stratified waters similar to that of the Gulf of Aqaba account for a significant portion (>50%) of the surface area of the ocean and therefore represent a significant fraction of the area available for carbon sequestration. Identifying the major factors that control phytoplankton growth, species abundance, and acclimation processes in these habitats is an important step in understanding their role in the global carbon cycle. Indeed, many studies have sought to address these issues, and recent findings suggest that the factors controlling Chl *a* levels and primary production may be different from those controlling cell division rate (Davey et al. 2008). Ecotypic variation among phytoplankton genera adds another level of complexity by helping to shape local and global phytoplankton distributions (Moore et al. 1998; Partensky et al. 1999; Rocap et al. 2003; Fuller et al. 2005; Johnson et al. 2006; Litchman and Klausmeier, 2008). Lindell et al. (2005) suggest that the dominance of certain phytoplankton taxonomic groups may be due to the ability of different ecotypes within those groups to thrive under specific environmental conditions, thereby allowing the entire population to acclimate to environmental stimuli. Clearly the factors influencing phytoplankton abundance in oligotrophic waters, and as a consequence the marine carbon cycle, are complex and more work is needed to fully elucidate phytoplankton growth dynamics in these regions. Moreover, observations of prolonged stratification (Karl et al. 2001) and projections of oligotrophic ocean expansion in response to global warming (Sarmiento et al. 1998; Matear and Hirst 1999; Sarmiento et al. 2004) highlight the importance of characterizing phytoplankton dynamics in these regions.

This study investigates survival strategies of naturally occurring phytoplankton communities in the oligotrophic, stratified Gulf of Aqaba in response to nutrient availability under various light regimes. We use field measurements from the euphotic zone of the Gulf of Aqaba, Red Sea before and during stratification at the onset of a spring bloom, as well as nutrient addition incubation experiments (at different light regimes) performed during the ultraoligotrophic summer season to: (1) assess shifts in phytoplankton community composition and photophysiology in response to the combined effects of nutrient and light availability; (2) identify key competition, acclimation, and nutrient uptake strategies of picoeukaryotes, *Synechococcus*, and *Prochlorococcus* during a bloom; and (3) estimate the contribution of picoeukaryotes, *Synechococcus*, and *Prochlorococcus* to total photosynthetic biomass during blooms as well as periods of sustained oligotrophy.

Materials and methods

Spring bloom monitoring

Water samples were collected from Station A (29°28′N, 34°55′E) in the Northern Gulf of Aqaba during the spring season when the water column transitions from mixed to stratified. Station A is located off shore in a deep (>700 m) open water region where the water column extends well below the euphotic zone; this permits analysis of an entirely pelagic euphotic zone. Depth profiles were taken on 12 March 2007 and 16 March 2007 using a sampling CTD-Rosette (SeaBird). Dissolved nutrient concentrations, Chl *a*, and flow cytometry measurements were taken as described below with the following modifications. For Chl *a* samples, filters were transferred immediately to 10 mL 90% acetone aboard the ship, and the pigment was extracted for 24 h at 4°C. Flow cytometry measurements were taken with a Becton Dickenson FACScan flow cytometer. On the basis of autofluorescence characteristics, cells were classified as picoeukaryotes or *Synechococcus*. Due to their relatively low autofluorescence compared to other

phytoplankton, *Prochlorococcus* cannot be reproducibly differentiated from other non-photosynthetic bacteria using the FACScan flow cytometer. Therefore, *Prochlorococcus* concentrations are not available from the field data set due to the fluorescence detection limit of the machine.

Simulated stratification experiment

A simulated stratification experiment was conducted to measure photoacclimation with higher time resolution than was possible for the field measurements described above. The experiment was designed to simulate the reversal of light limitation experienced by cells during the natural transition from mixing to stratification that occurs during the spring bloom. Surface water (1 m) was collected from a pier 20 m offshore on 12 March 2007 (prior to the spring bloom), and placed into 10 L sample-rinsed translucent polyethylene bottles (3 replicate bottles per treatment) 1 h before sunrise at the InterUniversity Institute for Marine Science (IUI) in Eilat, Israel. Water was pre-filtered through 20 μm mesh to remove grazers. Because nutrients were naturally abundant in sample waters as a consequence of deep mixing that preceded the experiment, it was not necessary to amend the sample water with additional nutrients to elicit a bloom response.

Sample bottles were incubated under light conditions designed to simulate either "shallow" (high light, HL) or "deep" (low light, LL) locations in the stratified water column. Bottles were incubated in an outdoor tank through which water from the Gulf circulated, and screening material was used to attenuate the sunlight intensity to which the bottles were exposed. In HL treatment, 50% light attenuation yielded maximum midday irradiance of ~1,000 μmol quanta m^{-2} s^{-1} and was equivalent to the upper 10 m of the euphotic zone of the Gulf during summer months. In the LL treatment, ~95% light attenuation yielded maximum midday irradiance of ~100 μmol quanta m^{-2} s^{-1} and was equivalent to the light intensity at approximately 80 m in the summer months [similar to the upper boundary of the deep chlorophyll maximum (D. Iluz, personal communication)]. No alteration of the natural light spectrum was done in the HL treatment. The light spectrum in the LL treatment was enriched in blue-green wavelengths using a tinted shade cloth to more closely resemble the natural light spectrum at 70–80 m depth. Comparison of spectra for unattenuated sunlight and light transmitted through the tinted cloth (each normalized to irradiance at 694 nm) showed that the cloth enriched the proportions of blue wavelengths at 443 and 490 nm by 280 and 210%, respectively, and green wavelengths at 510 and 555 nm by 190 and 150%, respectively, relative to the unfiltered visible spectrum.

The bottles were incubated for 2 days and aliquots were removed approximately every 3–4 h during the day for sampling. Flow cytometry samples were removed and processed as described below at each time point. Photosystem II (PSII) chlorophyll fluorescence measurements were taken by concentrating cells in the dark onto GF/F filters (Whatman) using a low pressure peristaltic pump. One liter of seawater was collected onto each sample filter. Chlorophyll fluorescence measurements were made with a WATER-PAM fluorometer and WinControl software (Heinz Walz GmbH). An automated program was run to determine the photochemical efficiency of PSII under dark-adapted (F_v/F_m) and light-adapted (Φ_{PSII}) states. (For detailed discussion of fluorescence parameter calculations and measurement techniques used in this study see Mackey et al. 2008). The program delivered the following light treatments and measuring pulses: 10 min dark adaptation and delivery of a saturating pulse to determine F_v/F_m, followed by 3 min exposure to actinic light at 100 μmol quanta m^{-2} s^{-1} and delivery of a saturating pulse to determine Φ_{PSII} at this light intensity (hereafter $\Phi_{PSII-100}$), followed by 3 min exposure to actinic light at 1,000 μmol quanta m^{-2} s^{-1} and delivery of a saturating pulse to determine Φ_{PSII} at this light intensity (hereafter $\Phi_{PSII-1,000}$). These actinic light exposure durations were determined empirically as sufficient for the cells to reach steady state.

Nutrient enrichment experiments

Nutrient enrichment experiments were conducted to ascertain phytoplankton responses to fertilization with exogenous inorganic nutrients (N and P) under incubation with HL and LL intensities simulating "shallow" and "deep" depths, respectively, within the water column. These experiments were designed to simulate bloom conditions resulting from delivery of new nutrients (e.g., via nitrogen fixation or atmospheric deposition) to a stratified, oligotrophic water column. Surface water was collected at 1 m depth from an offshore site in the Northern Gulf of Aqaba during two field excursions in September 2005 and October 2006 [note that community structure during these months is quite consistent from year to year (Lindell and Post 1995; Mackey et al. 2007)] Surface water was pre-filtered with 20 μm nylon mesh to remove grazers larger than 20 μm, collected into sample-rinsed translucent polyethylene bottles, and kept in the dark during transport (less than two hours) to the IUI facility. All incubations were performed with three replicate bottles per treatment.

The LL nutrient enrichment experiment was conducted in September 2005 and the HL nutrient enrichment experiment in October 2006. Light attenuation and incubation setup were performed exactly as described above for the simulated stratification experiment, using the same screening materials and incubation conditions. Phosphorus was added as 0.4 μmol L^{-1} sodium phosphate monobasic (hereafter PO_4),

and N was added as 7 μmol L^{-1} sodium nitrate (hereafter NO_3) or ammonium chloride (hereafter NH_4). Sample bottles contained 6 L water and were incubated for 4 days. The nutrient concentrations used in the enrichments were based on typical PO_4 and NO_3 concentrations for deep water from the Gulf (Mackey et al. 2007) because phytoplankton are naturally exposed to N and P at these levels following deep mixing and upwelling events. Nutrients were therefore added at an N:P ratio of 17.5:1, rather than the canonical Redfield ratio of 16:1, to better simulate natural bloom forming conditions.

Nutrient analyses

Total oxidized nitrogen (nitrate and nitrite, N + N) and soluble reactive phosphorus (SRP) concentrations were determined using colorimetric methods described by Hansen and Koroleff (1999), modified for a Flow Injection Autoanalyzer (FIA, Lachat Instruments Model QuickChem 8000). In all treatments not receiving sodium phosphate, SRP was pre-concentrated before analysis by a factor of about 20 using the magnesium co-precipitation (MAGIC) method (Karl and Tien 1992). Using standard additions, the recovery of inorganic P in this procedure was determined to be 100% and the blank was always below detection limits. The FIA was calibrated using standards prepared in low nutrient filtered seawater (summer surface water from the Gulf) over a range of 0–10 μmol L^{-1}. The precision of the methods used in this work is 0.05 μmol L^{-1} for N + N, and 0.02 μmol L^{-1} for SRP. The detection limit for these nutrients was 0.02 μmol L^{-1}.

Chlorophyll *a*

Daily samples for chlorophyll *a* (Chl *a*) determination were taken during both nutrient addition bioassays by filtering 200 mL aliquots onto Whatman GF/F glass fiber filters. In the HL nutrient enrichment experiment, samples were analyzed daily following extraction at 4°C in 90% acetone for 24 h in the dark. In the LL nutrient enrichment experiment, aliquots were removed from each bottle beginning at 3 pm each day, refrigerated overnight in the dark and filtered beginning at 10 am the following morning. These filters were placed within sterile 2 mL cryovials, stored at −80°C, and extracted for 24 h in 90% acetone (7 mL per sample) within 1 week. No significant effect of storage was observed based on comparisons between sample sets analyzed with and without storage; Chl *a* concentrations determined by the two methods were statistically indistinguishable. Fluorescence was measured with a Turner Fluorometer (Turner Designs 10-AU-005-CE) before and after acidification with 3.7% HCl and was converted to a Chl *a* concentration using a standard conversion method (JGOFS Protocols 1994). The standard error determined from triplicate samples was below 0.03 and 0.06 mg m^{-3} in the HL and LL nutrient enrichment experiments, respectively.

Flow cytometry

Aliquots were removed daily during the nutrient addition bioassays for flow cytometry, fixed with glutaraldehyde (final concentrations 0.1%) and stored at −80°C until analyzed on a FACSAria flow cytometer. Data analysis was performed using FlowJo software (TreeStar, Inc.). Picophytoplankton (photosynthetic cells <2 μm diameter) were classified as picoeukaryotes, *Prochlorococcus*, or *Synechococcus* on the basis of autofluorescence characteristics. Growth curves generated from the cell concentration data represent net growth for each group, i.e., growth minus mortality. Cell concentrations were determined by spiking samples with a known volume and concentration of 1 μm fluorescent yellow-green beads (Polysciences). The coefficient of variation for cell concentrations determined from triplicate samples was below 0.15 in both experiments. Mean and median cellular red fluorescence levels for picoeukaryotes, *Synechococcus*, and *Prochlorococcus* populations were estimated from the logarithmic signals collected on the Cy55-PE channel (488 nm excitation, 665 nm emission) of the flow cytometer.

Estimation of photosynthetic biomass

The contribution of each cell type to the overall picophytoplankton photosynthetic carbon biomass was estimated using the equation of Verity et al. (1992), which relates cell volume to carbon (C) biomass. Estimates of cell dimensions for *Prochlorococcus* (∼0.7 μm) and picoeukaryotes (∼1.5 μm) were based on light microscopy using a Nikon epifluorescent microscope at 400× magnification and from side-scatter measurements (a proxy for cell size) taken during flow cytometry of fixed cells collected in the field. Measurements fell within the typical range observed in other studies (Campbell et al. 1994). Both *Prochlorococcus* and picoeukaryotes were spherical, giving conversion factors of 98 and 708 fg C $cell^{-1}$, respectively. The conversion factor for *Prochlorococcus* is within the range of calculated estimates (29 and 124 fg C $cell^{-1}$) reported in other field studies (Partensky et al. 1999 and references therein; Zubkov et al. 2000; Grob et al. 2007), but is higher than an estimate of 49 fg C $cell^{-1}$ measured from laboratory cultures (Cailliau et al. 1996). Conversion factors for picoeukaryotes vary substantially due to the broader range of possible cell diameters; however, our estimate is within the range of calculated estimates (530–863 fg C $cell^{-1}$) for picoeukaryotes from the Pacific Ocean (Worden et al. 2004) with diameters

similar to those determined for this study. Microscopy revealed rod-shaped *Synechococcus* cells (dimensions ~0.8 to ~1.2 µm), with a composition of 279 fg C cell^{-1}, which is similar to previous estimates of ~250 fg C cell^{-1} (Kana and Glibert 1987; Campbell et al. 1994). Conversion factors were multiplied by cell concentration (determined from flow cytometry) to estimate photosynthetic biomass. While natural variability of phytoplankton cell size within the population introduces some error into our estimates, approximations of cellular C content from cell biovolume can inform productivity models if the potential for error is adequately considered when interpreting the results (Campbell et al. 1994; Worden et al. 2004).

Results

Spring bloom monitoring

To investigate phytoplankton responses to nutrient and light availability during the spring bloom, in situ monitoring of Chl *a,* flow cytometry, and nutrient draw down were carried out before and during the 2007 spring bloom. Figure 1 shows depth profile measurements of SRP, NO_3, Chl *a*, and *Synechococcus* and picoeukaryotes cell concentrations from the euphotic zone before (12 March 2007) and after (16 March 2007) stratification had begun to occur. Prior to stratification, 60 m is the typical depth at which light is attenuated to 1% of surface intensity (~20 µmol quanta m^{-2} s^{-1}) in the mixed Gulf water column (D. Iluz, personal communication). Because the water column was homogenously mixed down to >600 m on March 12 and the salinity profiles did not change over the course of the sampling (data not shown), we conclude that the observed changes in the water column during this period are due to stratification rather than introduction of different water masses to the euphotic zone via other physical processes in the water column. This interpretation is consistent with the current understanding of the circulation in the Gulf (Wolf-Vetch et al. 1992). NO_3 and SRP levels were relatively homogenous (1.7 and 0.11 µmol L^{-1}, respectively) throughout the mixed water column (on March 12) (Fig. 1a, b), whereas drawdown was evident in the upper 50 m of the stratified water column (March 16). Based on temperature profiles, the surface mixed layer was approximately 25 m on March 16 during stratification. In these profiles, NO_3 levels were 0.8 µmol L^{-1} at the surface and increased to 1.8 µmol L^{-1} at depth, and SRP levels were 0.05 µmol L^{-1} at the surface and increased to 0.08 µmol L^{-1} at depth (Fig. 1a, b).

A Chl *a* maximum (1.26 mg m^{-3}) was observed in the stratified water column at 20 m depth, whereas the Chl *a* distribution was homogenous throughout the mixed water column on 12 March (0.2 mg m^{-3}; Fig. 1c). In the mixed profile, picoeukaryotes were present at approximately 3,000–4,000 cells mL^{-1}, and *Synechococcus* were present at approximately 2,000–3,000 cells mL^{-1} throughout the euphotic zone (Fig. 1d, e). The maximum picoeukaryote cell concentration in the stratified profile on 16 March occurred at 60 m (17,000 cells mL^{-1}), and another smaller peak was apparent near 120 m (11,000 cells mL^{-1}). In contrast, *Synechococcus* cell concentration profiles showed two small peaks at 20 m (7,000 cells mL^{-1}) and 100 m (8,000 cells mL^{-1}) that were offset from the picoeukaryote peaks (Fig. 1d, e). Cell abundances of *Prochlorococcus* and larger phytoplankton were not available for these casts, so it is not possible to know their vertical distributions. However, in typical years in the Gulf, *Prochlorococcus* numbers remain relatively constant throughout the spring bloom (Lindell and Post 1995; A. Paytan unpublished data). The profiles of Chl

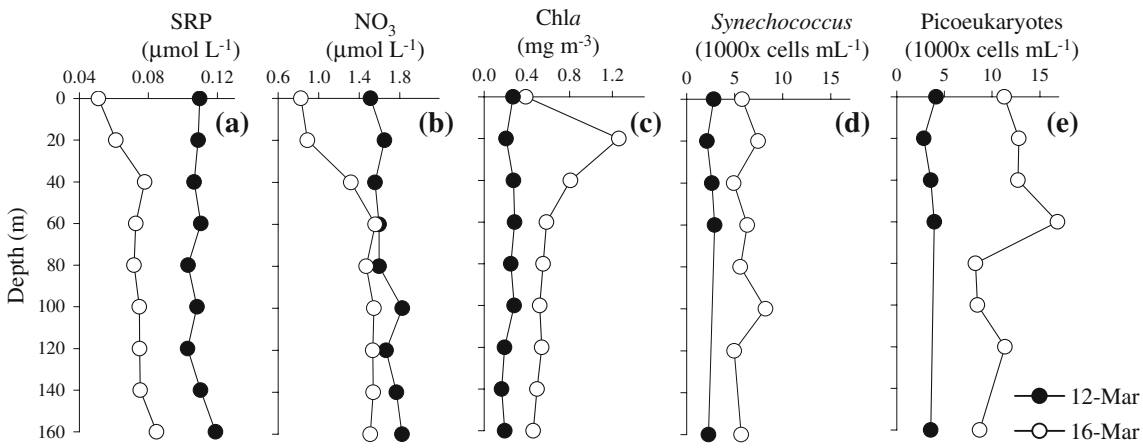

Fig. 1 Depth profiles of **a** SRP, **b** NO_3, **c** Chl *a*, **d** *Synechococcus* cell concentration, and **e** picoeukaryote cell concentration for the mixed (*closed circles*) and stratified (*open circles*) water columns

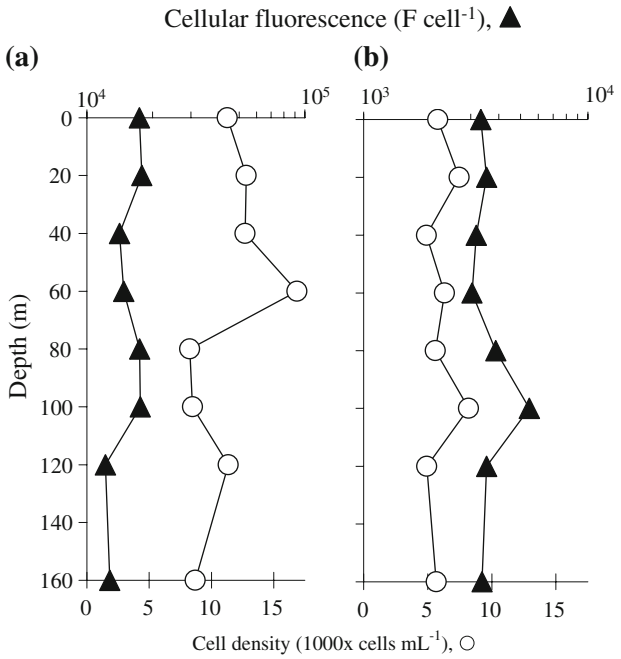

Fig. 2 Cellular fluorescence (and corresponding cell densities) for **a** picoeukaryotes, and **b** *Synechococcus* on 16 March

a would include input from both *Prochlorococcus* and larger cells. In particular, inputs from larger cells in surface waters (Mackey et al. 2007) may explain the Chl *a* peak at 20 m that is offset from *Synechococcus* and picoeukaryote peaks.

On 16 March, picoeukaryote cellular fluorescence showed no systematic trends with depth (Fig. 2a), whereas *Synechococcus* cellular fluorescence was higher for cells at 100 m, the depth of the deep cell maximum, than at 20 m, the depth of the surface maximum (Fig. 2b).

Figure 3a shows the ratio of NO_3 to PO_4 (NO_3:PO_4) calculated from NO_3 and SRP measurements at each depth for the mixed and stratified casts. A dashed line at 16 indicates the value of the Redfield ratio (Redfield et al. 1963). In the mixed profile the NO_3:PO_4 ratio was approximately 16 throughout the water column except at the surface where it was approximately 14. In the stratified cast the NO_3:PO_4 ratio was also near 14 in surface waters, but increased to approximately 20 at greater depths. The drawdown of NO_3 and SRP determined from changes in water nutrient concentrations over the period of sampling are shown in Fig. 3b and c, respectively, and show that larger decreases in the concentrations of both nutrients (i.e., greater drawdown) occurred in the upper 50 m of the euphotic zone. The ratios of NO_3 and PO_4 drawdown (Fig. 3d) are close to the Redfield ratio of 16 in the surface, but decrease drastically at 60 m (NO_3:PO_4 of approximately 1) before increasing again with depth but still remaining below Redfield ratios (NO_3:PO_4 between 5 and 10). These uptake ratios are indicative of the ratios at which these nutrients are removed from the water during the sampling period, e.g., through biological immobilization or export (Eppley and Peterson 1979) assuming (1) the inventory of nutrients (i.e., dissolved plus particulate) in the euphotic zone was constant over the time of our sampling, and (2) that there was no vertical or horizontal flux of nutrients due to lateral diffusion or isopycnal mixing. These assumptions are reasonable for the northern Gulf, given that the north-south gradient in surface waters nutrients is low throughout the year (Klinker et al. 1977; A. Paytan, unpublished data), the short time scale of our calculation (four days), and given that a one dimensional mixed layer model accurately describes the convective/advective water balance at the northern end of the Gulf where our sampling was conducted (Wolf-Vetch et al. 1992).

Simulated stratification experiment

The picophytoplankton community responded similarly to incubation under HL and LL conditions in the simulated

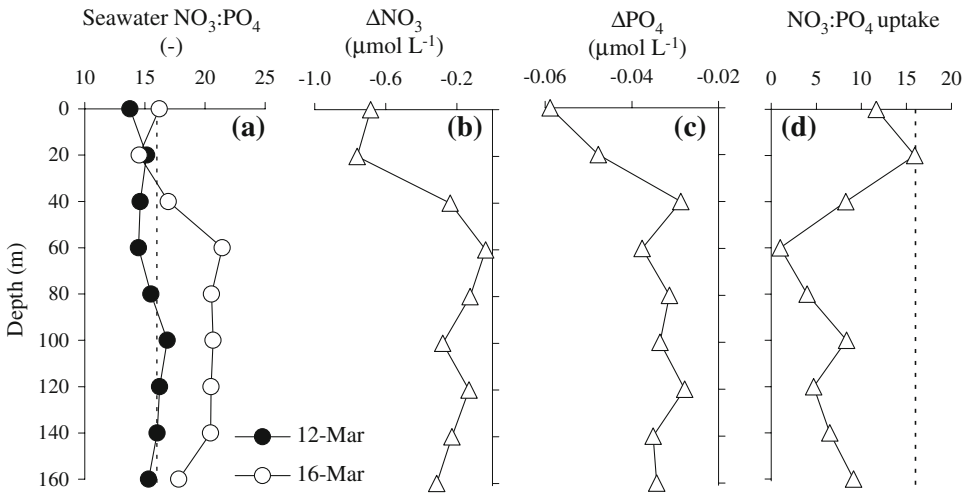

Fig. 3 Depth profiles of **a** seawater NO_3:PO_4 ratios for the mixed (*closed circles*) and stratified (*open circles*) water columns; and the changes in **b** NO_3 concentration; **c** PO_4 concentration; and **d** NO_3:PO_4 uptake ratios during the transition from mixing to stratification

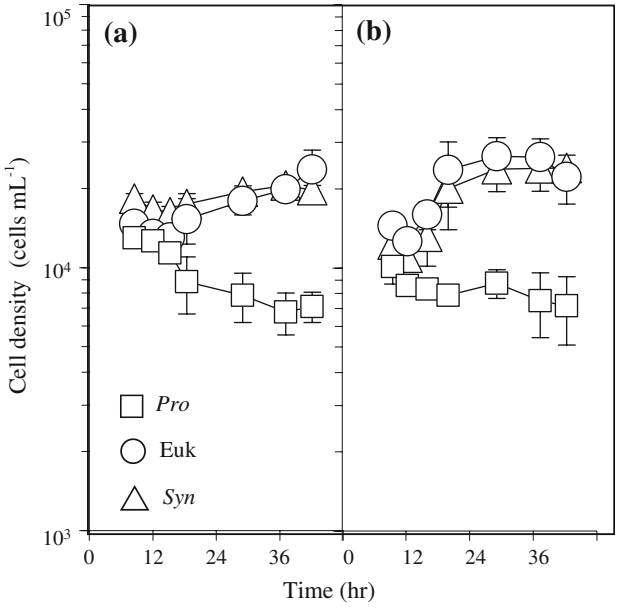

Fig. 4 Cell concentration time series for the **a** high light treatment and **b** low light treatment in the simulated stratification experiment. *Error bars* represent SE of the mean of triplicate measurements and are contained within the symbol when not visible

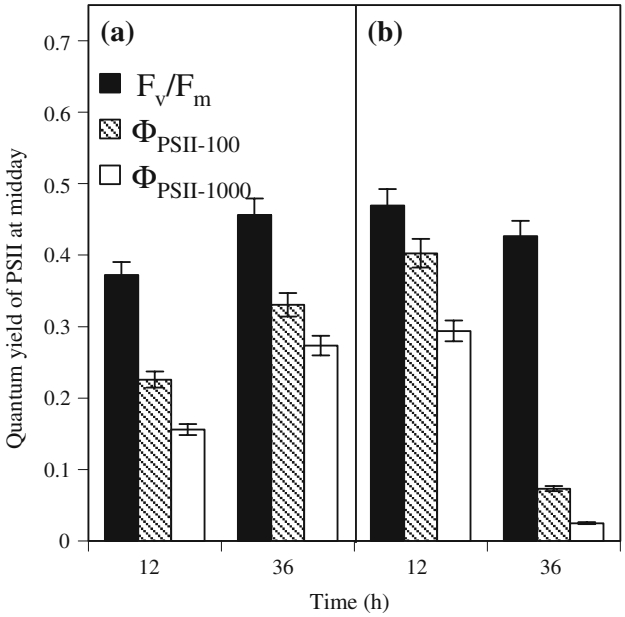

Fig. 5 Photochemical efficiency of PSII at midday in the **a** high light treatment and **b** low light treatment in the simulated stratification experiment. Samples were taken at noon on the first and second day of the experiment (hours 12 and 36, respectively). The parameter $\Phi_{PSII-100}$ is the photochemical efficiency of PSII during 100 µmol quanta m^{-2} s^{-1} actinic light, and $\Phi_{PSII-1,000}$ is the photochemical efficiency of PSII during 1,000 µmol quanta m^{-2} s^{-1} actinic light. *Error bars* represent SE of the mean of triplicate measurements

stratification experiment. The initial population was composed of picoeukaryotes (14,500 cells mL^{-1}), *Synechococcus* (19,600 cells mL^{-1}), and *Prochlorococcus* (12,900 cells mL^{-1}). Picoeukaryotes and *Synechococcus* increased in abundance, and *Prochlorococcus* decreased in abundance, over 2 days in both the HL (Fig. 4a) and LL (Fig. 4b) treatments.

Midday (noon) PSII fluorescence measurements for the simulated stratification experiment are shown in Fig. 5. F_v/F_m and Φ_{PSII} measurements increased slightly compared to initial midday levels within one day of incubation in the HL treatment (Fig. 5a). In contrast, F_v/F_m measurements remained relatively stable in the LL treatment (Fig. 5b), while both $\Phi_{PSII-100}$ and $\Phi_{PSII-1,000}$ decreased by over 75% by midday on the second day of the experiment (i.e., hour 36) compared to initial midday levels (hour 12).

Nutrients uptake in nutrient enrichment experiments

Ambient seawater nutrient concentrations measured prior to the addition of experimental amendments in the HL and LL nutrient enrichment experiments are shown in Table 1, and reflect the oligotrophic character of surface waters in the Gulf during the stratified season in which the experiments were conducted in both years. The nutrient data from the HL and LL nutrient enrichment experiments are discussed semi-quantitatively because nutrient levels in several of the treatments were close to detection limits and differences between treatments were, in some cases, close to the analytical precision. However, while statistical statements are not made, the conclusions herein are nevertheless based on comparison of averages and standard deviations of triplicate samples. In the HL nutrient enrichment experiment, SRP decreased in treatments receiving PO_4 together with added sources of N (i.e., NO_3 or NH_4, Table 1). The changes in SRP concentration for all other treatments were not substantially different from the control. Decreases in inorganic N (N_i) were observed in treatments with NO_3 and PO_4 together, and NO_3 alone. In the LL nutrient enrichment experiment, SRP concentrations similarly decreased only in treatments with PO_4 when NH_4 or NO_3 were also added. All other treatments showed slight decreases in SRP concentration but were not substantially different from the control. N_i decreased following treatment with NO_3 and PO_4 together but not with NO_3 alone, while all other changes were similar to the control (Table 1).

Chlorophyll *a* in nutrient enrichment experiments

Chl *a* levels were monitored daily throughout the HL and LL nutrient enrichment experiments as described above. Figure 6a shows the Chl *a* time series in the HL nutrient enrichment experiment. The initial Chl *a* level was 0.15 mg m^{-3}. The Chl *a* concentration in the control decreased to 0.02 mg m^{-3} by the final day of the experiment.

Table 1 Nutrient data

Treatment	Initial nutrient levels after nutrient addition treatment		Changes in nutrient levels	
	N_i	SRP	ΔN	ΔP
HL experiment				
NH_4,PO_4	7.08 ± 0.01	0.43 ± 0.01	0.07	−0.14
NO_3,PO_4	7.08 ± 0.12	0.43 ± 0.01	−1.41	−0.08
NO_3	7.08 ± 0.11	0.03 ± 0.00	−0.22	0.01
NH_4	7.08 ± 0.03	0.03 ± 0.00	0.00	0.00
PO_4	0.08 ± 0.00	0.43 ± 0.00	−0.02	−0.01
Control	0.08 ± 0.00	0.03 ± 0.00	0.02	0.00
LL experiment				
NH_4,PO_4	7.13 ± 0.03	0.44 ± 0.02	0.07	−0.16
NO_3,PO_4	7.13 ± 0.05	0.44 ± 0.01	−1.18	−0.16
NO_3	7.13 ± 0.10	0.04 ± 0.01	0.05	−0.02
NH_4	7.13 ± 0.01	0.04 ± 0.01	0.01	−0.01
PO_4	0.13 ± 0.03	0.44 ± 0.01	−0.05	−0.07
Control	0.13 ± 0.02	0.04 ± 0.01	0.15	−0.02

Initial inorganic N (N_i) and SRP levels following nutrient additions were estimated as the sum of the mean background concentrations of N_i and SRP at day zero plus the calculated amount of N_i or SRP added from the nutrient addition treatments. Standard errors are given following the means. Changes in NO_3 and NO_2 (ΔN) and P (ΔP) concentrations between these initial levels and levels at day 4 of the LL and HL nutrient enrichment experiments are also shown. Negative values indicate consumption and positive values indicate production of nutrients throughout the experiments (μmol L^{-1}). Values are averages of triplicate samples

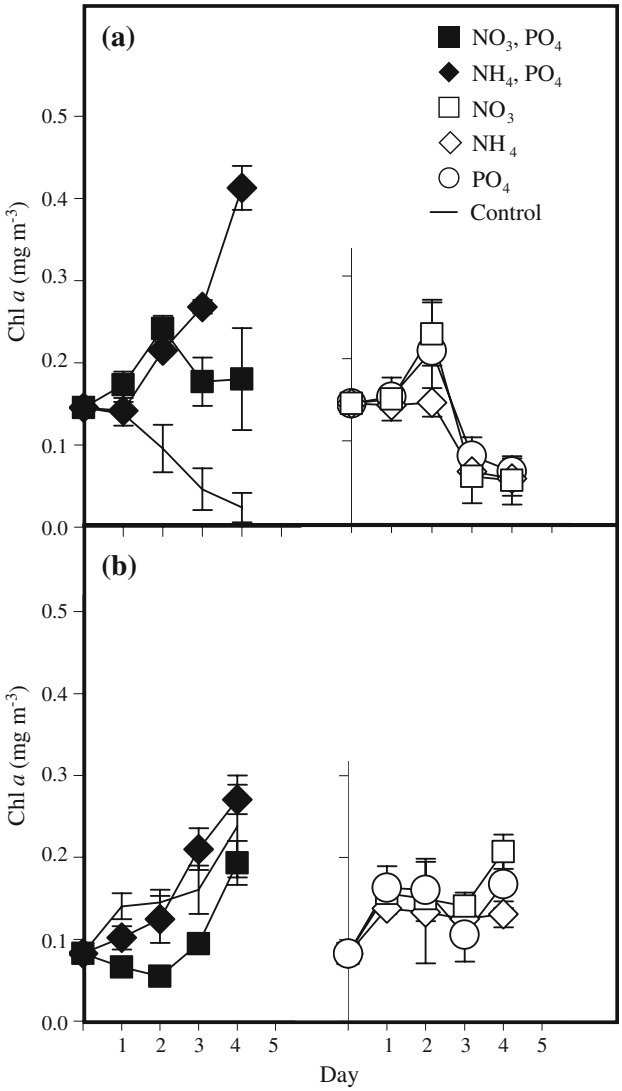

Fig. 6 Chlorophyll *a* concentrations for the **a** high light and **b** low light nutrient enrichment experiments. *Error bars* represent SE of the mean of triplicate measurements

The largest increase in Chl *a* was observed in the treatment receiving NH_4 and PO_4 together (0.41 mg m^{-3}), which showed a greater than 3-fold increase over the initial level and a 17-fold increase relative to the control on the final day (Fig. 6a). Treatment with PO_4 and NO_3 together yielded a smaller but measurable increase (0.18 mg m^{-3}). By the final day of the experiment, treatments of PO_4, NO_3, or NH_4 alone (i.e., single nutrient amendments) showed decreased Chl *a* levels relative to the initial level (0.06, 0.05, and 0.05 mg m^{-3}, respectively) that were similar or slightly higher than that of the control (Fig. 6a). The standard error determined from triplicate samples in the HL nutrient enrichment experiment was below 0.03 mg m^{-3} for all treatments.

In the LL nutrient enrichment experiment all treatments (including the control) resulted in elevated Chl *a* levels relative to the initial level (0.08 mg m^{-3}); however, none of the nutrient addition treatments resulted in Chl *a* levels that were significantly higher than the control (0.24 mg m^{-3}) by day 4 of the experiment (Fig. 6b). Treatment with PO_4, NH_4, or NO_3 alone resulted in twofold increases in Chl *a* compared to the initial values (0.17, 0.13, and 0.21 mg m^{-3}, respectively). Treatments of PO_4 given together with either NH_4 or NO_3 showed Chl *a* levels above initial levels but similar to the control (0.27 and 0.19 mg m^{-3}, respectively). The standard error determined from triplicate samples in the LL nutrient enrichment experiment was below 0.06 mg m^{-3} for all treatments.

Flow cytometry cell counts in nutrient enrichment experiments

To determine how picophytoplankton community composition changes in response to nutrients and light availability, we measured the changes in cell densities of picoeukaryotes, *Synechococcus*, and *Prochlorococcus* in the HL and LL nutrient enrichment experiments using flow cytometry. Figure 7 shows picoeukaryotes, *Synechococcus*, and *Prochlorococcus* cell concentrations throughout the course

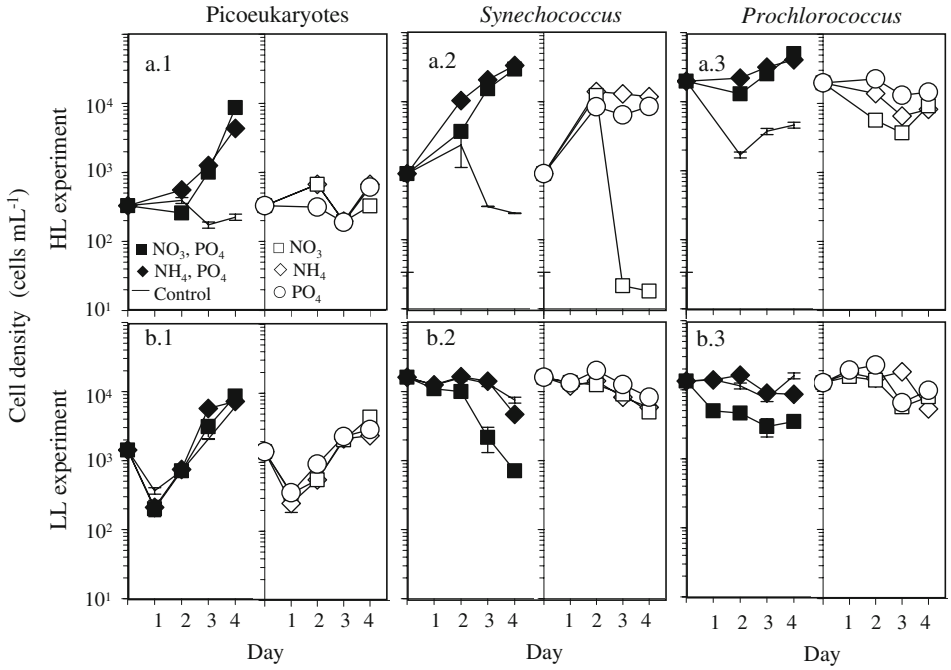

Fig. 7 Cell concentration time series for the **a** high light and **b** low light nutrient enrichment experiments for (*a.1*) picoeukaryotes; (*a.2*) *Synechococcus*; and (*a.3*) *Prochlorococcus*. The coefficient of variation for triplicate samples was below 0.15; error bars (*SE*) are contained within symbols where not visible

of the HL and LL nutrient enrichment experiments, and Table 2 shows the final concentrations and the relative increases for each treatment compared to the control on the last day of the experiment. (The coefficient of variation for cell concentrations determined from triplicate samples was below 0.15 for both the HL and LL nutrient enrichment experiments.) *Prochlorococcus* (Table 2, Fig. 7a.3) was the dominant cell type at the start of the HL experiment, followed by *Synechococcus* (Table 2, Fig. 7a.2) and picoeukaryotes (Table 2, Fig. 7a.1), whereas *Synechococcus* (Fig. 7b.2) was the dominant cell type at the start of the LL nutrient enrichment experiment, followed by *Prochlorococcus* (Fig. 7b.3) and picoeukaryotes (Fig. 7b.1). In the HL nutrient enrichment experiment, the greatest increases in picoeukaryote and *Synechococcus* cell numbers (1–2 orders of magnitude increase compared to control) occurred in treatments receiving N and P together. *Prochlorococcus* cell concentrations also increased relative to the control for treatments receiving PO_4 together with either NO_3 or NH_4, but were less than an order of magnitude greater than the control and remained similar to initial levels (Table 2, Fig. 7a). In contrast, cell concentration measurements for all three organisms were typically less than or comparable to that of the control for treatments in the LL nutrient enrichment experiment (Table 2, Fig. 7b).

Photosynthetic biomass in nutrient enrichment experiments

To estimate the relative contributions of picoeukaryotes, *Synechococcus*, and *Prochlorococcus* to primary production during a bloom, we estimated changes in the photosynthetic biomass of each of these groups during the HL and LL nutrient enrichment experiments. Figure 8 shows the change from initial picophytoplankton photosynthetic biomass levels to those on day 4 of each experiment for picoeukaryotes, *Synechococcus*, and *Prochlorococcus*. There were no substantial net changes in picophytoplankton photosynthetic biomass for any treatments relative to initial levels in the LL nutrient enrichment experiment, where the photosynthetic biomasses for all treatments were similar to initial levels (6.8 mg C m^{-3}). In these low light samples, the contribution from *Prochlorococcus* and *Synechococcus* decreased in all treatments except for the control, while the contribution of picoeukaryotes increased for all treatments (Fig. 8b). The HL nutrient enrichment experiment (Fig. 8a), in contrast, showed net increases in picophytoplankton photosynthetic biomass relative to initial levels (2.4 mg C m^{-3}) when N and P were added together (16.3 and 19.3 mg C m^{-3} for treatments $NH_4 + PO_4$ and $NO_3 + PO_4$, respectively), similar levels following additions of PO_4 (4.2 mg C m^{-3}) or NH_4 (4.6 mg C m^{-3}), and decreased levels for the control and only NO_3 treatments (1.1 mg C m^{-3}).

Flow cytometry cellular fluorescence in nutrient enrichment experiments

To assess photoacclimation in the picoeukaryote, *Synechococcus*, and *Prochlorococcus* populations HL and LL nutrient enrichment experiments we estimated cellular fluorescence, a proxy for cellular pigment content (Sosik et al. 1989; Li et al. 1993), from flow cytometry. Figure 9 shows the mean and median red fluorescence of cells on day 0 and 4 of the LL nutrient enrichment experiment. By day 4 of the experiment, red fluorescence increased twofold

Table 2 Absolute cell concentrations (cells mL^{-1}) of each picophytoplankton cell type on day 4 of the HL and LL nutrient enrichment experiments as determined by flow cytometry

Treatment	Picoeukaryotes (cells mL^{-1})	*Synechococcus* (cells mL^{-1})	*Prochlorococcus* (cells mL^{-1})	All picophytoplankton (cells mL^{-1})
HL experiment				
NH_4,PO_4	4,300 (1,433, 2,150%)	33,300 (3,700, 16,650%)	40,400 (205, 878%)	78,000 (373, 1560%)
NO_3,PO_4	8,700 (2,900, 4,350%)	29,600 (3,289, 14,800%)	49,800 (253, 1083%)	88,100 (422, 1762%)
PO_4	600 (200, 300%)	8,500 (944, 4,250%)	14,500 (74, 315%)	23,600 (113, 472%)
NH_4	700 (233, 350%)	11,800 (1,311, 5,900%)	8,200 (42, 178%)	20,700 (99, 414%)
NO_3	300 (100, 150%)	20 (2, 10%)	9,200 (47, 200%)	9,500 (45, 190%)
Control	200 (67, 100%)	200 (22, 100%)	4,600 (23, 100%)	5,000 (24, 100%)
Day zero	300 (100, 150%)	900 (100, 450%)	19,700 (100, 428%)	20,900 (100, 418%)
LL experiment				
NH_4,PO_4	7,200 (514, 120%)	4,700 (29, 62%)	8,600 (64, 54%)	20,500 (66, 69%)
NO_3,PO_4	8,600 (614, 143%)	700 (4, 9%)	3,500 (26, 22%)	12,800 (41, 43%)
PO_4	3,000 (214, 50%)	8,300 (52, 111%)	10,400 (78, 66%)	21,700 (70, 74%)
NO_3	4,500 (321, 75%)	5,000 (31, 66%)	8,400 (63, 53%)	17,900 (58, 61%)
NH_4	2,400 (171, 40%)	5,900 (37, 79%)	5,600 (42, 35%)	13,900 (45, 47%)
Control	6,000 (429, 100%)	7,500 (47, 100%)	15,900 (119, 100%)	29,400 (95, 100%)
Day zero	1,400 (100, 23%)	16,100 (100, 213%)	13,400 (100, 84%)	30,900 (100, 105%)

The total concentration of all picophytoplankton cells is shown in the last column, and concentrations at day zero are also shown. Percentage of cells in each experimental treatment relative to time zero and control are given in parentheses

in the picoeukaryote population (Fig. 9a) and fivefold in the *Synechococcus* population (Fig. 9b) regardless of nutrient treatment. Red fluorescence in the *Prochlorococcus* population was similar on day 4 and day 0 in all nutrient treatments (Fig. 9c). Red fluorescence levels in cell populations on day 4 of the HL nutrient enrichment experiment did not show any systematic trends relative to day 0 levels (data not shown).

Discussion

Nutrient limitation refers to the slowing or cessation of photosynthetic biomass production in response to low availability of an essential nutrient. Together, the Redfield ratio and Liebig's law of the minimum have been used in oceanography to identify nutrient limitation at the phytoplankton community level (von Liebig 1840; Redfield et al. 1963). These interpretations are based on the assumption that phytoplankton communities assimilate macronutrients at a constant ratio, and therefore deviations from such ratios indicate limitation by one nutrient over another. However, nutrient co-limitation can occur in oligotrophic waters (Seppala et al. 1999; Mills et al. 2004) where addition of one nutrient may rapidly induce limitation for another nutrient as soon as limitation by the former is relieved. In these cases phytoplankton communities are effectively co-limited because addition of both nutrients is required to elicit substantial increases in biomass. Therefore, while only one nutrient can be physiologically limiting at a given point in time, more than one nutrient may effectively co-limit phytoplankton on ecologically relevant time scales. In this study, the term "co-limitation" refers to inhibition of photosynthetic biomass production, rather than to the physiological nutrient status of a cell or group of cells per se.

In the stratified Gulf of Aqaba, N and P co-limit the phytoplankton community. Under irradiances similar to levels that would be seen in the upper euphotic zone (HL nutrient enrichment experiment), additions of inorganic N and P together resulted in elevated Chl *a* levels, while addition of N or P independently did not (Fig. 6a) (Fe, Si, and other trace metal concentrations are elevated in the waters of the Gulf (Longhurst 1998; Chase et al. 2004; Chen et al. 2007), thus limitation by these elements was not considered here). Indeed, estimates of picophytoplankton photosynthetic biomass for the HL nutrient enrichment experiment showed that the largest increases occurred when N and P were added simultaneously (Fig. 8a). We therefore conclude that during the time of our experiments, oligotrophic conditions in surface waters (Table 1) caused co-limitation of the phytoplankton community by N and P when light was not limiting (Figs. 6a, 8a; see also Labiosa 2007). This type of limitation is referred to as "multi-nutrient co-limitation," and results when the levels of two or more nutrients are depleted beyond the levels required for cellular uptake (Arrigo 2005 and references therein).

The HL nutrient enrichment experiment showed that the phytoplankton community did not respond homogenously

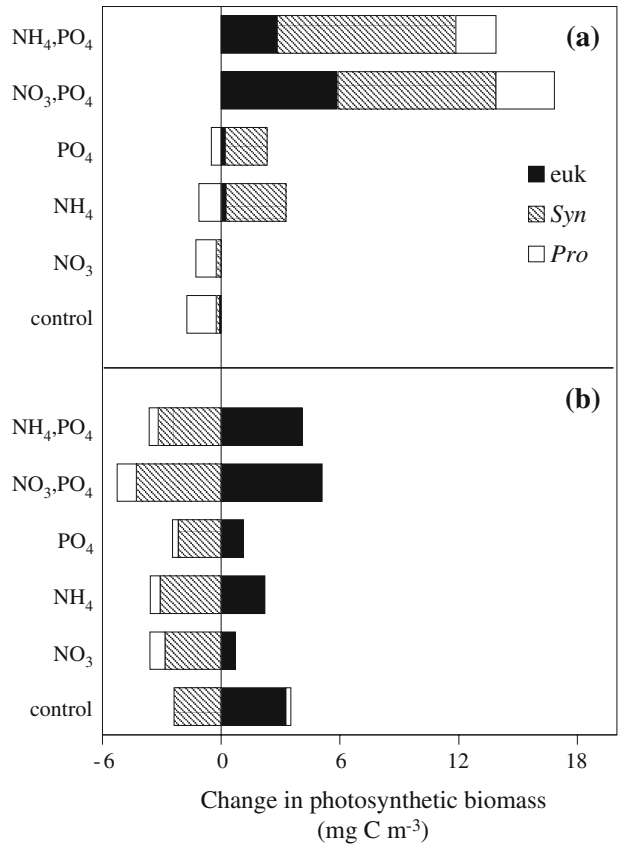

Fig. 8 Estimates of picophytoplankton photosynthetic biomass on the final day of the **a** high light and **b** low light nutrient enrichment experiments contributed by picoeukaryotes (*black bars*), *Synechococcus* (*hatched bars*), and *Prochlorococcus* (*white bars*)

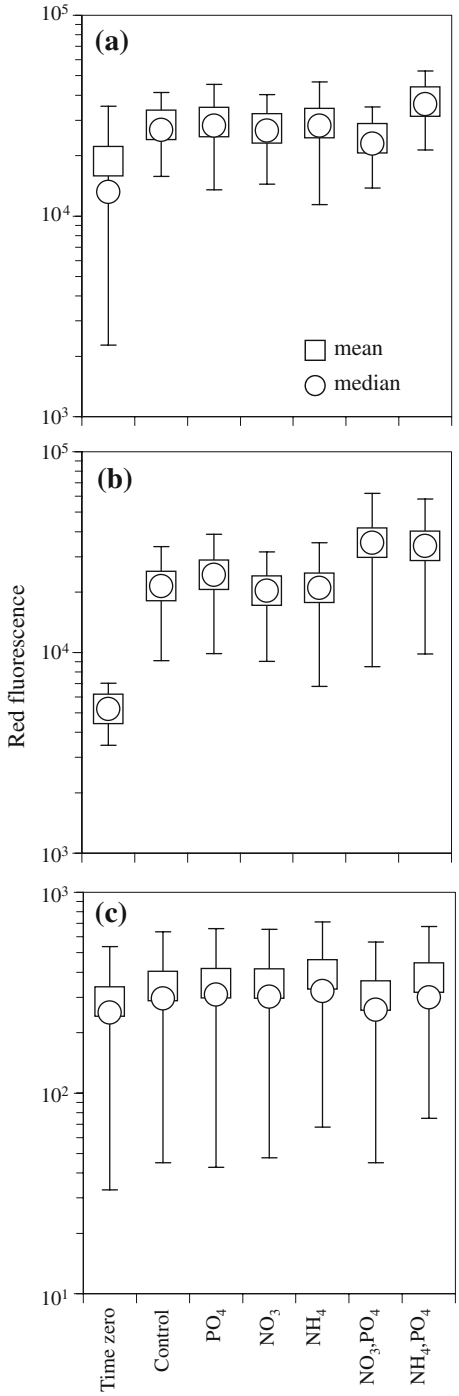

Fig. 9 Cellular fluorescence during the LL nutrient enrichment experiment for **a** picoeukaryotes, **b** *Synechococcus*, and **c** *Prochlorococcus*. Plots show fluorescence per cell on day 0 for the initial populations, and on day 4 for each treatment and the control. *Error bars* show the standard deviations of the cellular fluorescence distributions

to nutrient additions under sufficient irradiances. Cell concentration measurements indicated that picoeukaryotes and *Synechococcus* responded to nutrient additions during the 4-day incubation period, whereas *Prochlorococcus* (the dominant organism at the start of our sampling) did not (Fig. 7a). Therefore, the majority of the photosynthetic biomass produced following addition of N and P was attributable to picoeukaryotes and *Synechococcus* (Fig. 8a). Similar results were observed in the simulated stratification experiment, where picoeukaryote and *Synechococcus* populations bloomed over a 2 day period when light limitation was reversed (Fig. 4). In this study, we focused on picophytoplankton bloom dynamics; we note, however, that larger cells also contribute to blooms components in the Gulf of Aqaba (Lindell and Post 1995; Mackey et al. 2007), contributing additional photosynthetic biomass. Our results indicate that when growth limiting nutrients become available and light is not limiting, blooms of specific subpopulations within the overall phytoplankton community can yield substantial increases in photosynthetic biomass as a result of their rapid growth responses.

The relatively rapid increase in the cell densities of picoeukaryotes and *Synechococcus* when N and P were provided together in the HL nutrient enrichment (Fig. 7a.1, a.2), and the simulated stratification (Fig. 4a, b) experiments suggested that these phytoplankton have a "bloomer" growth strategy (Margalef 1978; Smayda and Reynolds 2001; Klausmeier et al. 2004; Labiosa 2007; see Arrigo

2005 for discussion of terminology). This growth response is similar to the in situ changes that occurred as the water column started to stratify and nutrient and light limitation are reversed, which show that the cell concentrations of picoeukaryotes (and to a lesser extent *Synechococcus)* increase two to threefold in the upper euphotic zone over a period of days (Fig. 1d, e). Moreover, picoeukaryotes were not numerically dominant in day zero samples, indicating that they were unable to remain abundant during prolonged oligotrophic summer conditions (Fig. 7a.1, b.1; Lindell and Post 1995; Labiosa 2007; Mackey et al. 2007), as would be expected for a bloomer strategist.

By contrast, *Prochlorococcus* was abundant in day zero samples and showed no rapid, substantial growth responses regardless of nutrient additions when light was not limiting in the HL nutrient enrichment experiment (Fig. 7a.3, b.3; see also Labiosa 2007). Similar results were observed in the simulated stratification experiment, where the *Prochlorococcus* population declined slightly over a 2 day period when light limitation was reversed but nutrients were still available from deep winter mixing. These data may suggest that *Prochlorococcus* adopts a "survivalist" growth strategy, enabling them to sustain growth during periods of low nutrient levels, possibly owing to lower growth rates, lower nutrient requirements (Bertilsson et al. 2003; Fuller et al. 2005; Van Mooy et al. 2006) and high affinity nutrient acquisition systems (Scanlan and Wilson 1999) compared to other picophytoplankton. Indeed, Lindell and Post (1995) observed that *Prochlorococcus* became the dominant phytoplankter in the Gulf only after several months of stratification and prolonged nutrient depletion had caused marked decreases in picoeukaryote and *Synechococcus* numbers.

However, while this pattern of succession is typical during most years in the Gulf (Israel National Monitoring Project, Eilat, http://www.iui-eilat.ac.il/NMP/, unpublished data), *Prochlorococcus* was a significant contributor to the spring bloom in 1999 (Fuller et al. 2005), suggesting that it may share characteristics of bloom strategists as well. Specifically the hydrographic and chemical conditions (stability and duration of the stratification, amount of nutrients injected, temperature and light intensities etc.) during bloom events and the related variable responses of *Prochlorococcus* should be evaluated in more detail. In the simulated stratification experiment, the lack of *Prochlorococcus* growth could be a function of the antecedent conditions of the sample water, rather than a function of a survivalist growth strategy. In contrast to the in situ monitoring at station A (a location in open water away from the coastal shelf), this experiment used water collected closer to shore. The higher concentrations of *Synechococcus* and picoeukaryotes in the time zero samples (Fig. 4) compared to the surface water from station A from the same day (12 March, Fig. 1) suggests that growth conditions varied slightly between the locations; coastal stratification may have preceded stratification at station A due to faster warming in shallower water, thereby accelerating the bloom. Therefore, it is possible that the relatively high *Prochlorococcus* concentrations in time zero samples could indicate that the growth response of these cells had already occurred and reached steady state before our sample water was collected, even though *Synechococcus* and picoeukaryotes continued to bloom for the duration of the experiment. Alternately, the size of the seed population of competent high light ecotypes of *Prochlorococcus* (rather than growth strategy) could explain the growth response (Fuller et al. 2005). Therefore, while *Prochlorococcus* has optimized its growth at lower nutrient levels and is abundant in nutrient poor waters (Partensky et al. 1999and references therein), more work is needed to accurately characterize *Prochlorococcus* growth strategies, particularly with respect to ecotypic diversity.

In addition to identifying multi-nutrient co-limitation in the phytoplankton community and the species specific response to alleviation of this co-limitation, our results also suggested that the Redfield ratio represents an average value of species-specific N:P ratios in the Gulf of Aqaba, as observed in other areas of the ocean (Elser et al. 1996). A model by Klausmeier et al. (2004) reports a range of phytoplankton elemental stoichiometries (N:P) of 8.2 to 45 depending on environmental conditions, and notes that variability occurs between and within species due to genetic diversity and physiological flexibility. The range of N:P ratios reported for *Synechococcus* (13.3-33.2) and *Prochlorococcus* (15.9-22.4) (Bertilsson et al. 2003; Heldal et al. 2003) demonstrate the flexibility in phytoplankton elemental stoichiometries in oligotrophic marine environments when faced with variable nutrient availability.

Our study demonstrates that the physiological flexibility with which nutrients are assimilated into photosynthetic biomass is important for phytoplankton dynamics during the spring bloom, where phytoplankton regulate and maintain their elemental stoichiometries via preferential uptake of certain nutrients. Phytoplankton with a "bloomer" growth strategy tend toward lower N:P ratios (Elser et al. 1996). Nutrient stoichiometries in the water column were close to Redfieldian prior to stratification (Fig. 3a). During the transition from mixing to stratification, the net removal of NO_3 and PO_4 from the water continued at near-Redfield ratios in the surface (likely reflecting a contribution from larger phytoplankton cells not counted in our analysis), whereas bulk uptake ratios were lower (<10) in the middle and lower euphotic zone (Fig. 3d) where *Synechococcus* and picoeukaryotes dominated (Fig. 1d, e). Moreover, the ratio reached a minimum value of 1 at 60 m, coinciding with the picoeukaryote maximum identified in Fig. 1e.

These results showed that as stratification progressed, more P was taken up overall relative to N than would be predicted by the Redfield ratio, possibly indicating luxury P uptake. These trends were consistent with the phytoplankton growth observed during the bloom (Fig. 1d, e), which favor resource partitioning toward P-rich cellular assembly machinery, including ribosomes, high energy adenylates, and nucleic acids (Elser et al. 1996, 2000; Geider and La Roche 2002). While this preferential uptake has been suggested based on theory and laboratory experiments, our field results also support it by relating changes in phytoplankton community composition (i.e., bloomers vs. survivalists) to nutrient uptake ratios.

The term "photoacclimation" encompasses the phenotypic changes that occur in an organism in response to changes in environmental factors affecting photosynthesis (MacIntyre et al. 2002). The photochemical yield of PSII is a measure of photoacclimation because it shows how efficiently PSII is able to use light to drive photochemistry. F_v/F_m, which is the maximum photochemical efficiency of PSII in dark adapted cells, is a measure of the number of functional PSII reaction centers, and tends to reach its lowest values at midday due to photoinhibition (Mackey et al. 2008). Φ_{PSII} is a measure of the photochemical efficiency of PSII under a given background (actinic) light intensity. It differs from F_v/F_m in that some of the functional PSII reaction centers are in the reduced state due to exposure to light at the time of measurement. In the HL treatment, an increase in Φ_{PSII} under both 100 and 1,000 µmol quanta m^{-2} s^{-1} shows that after only one and a half days of incubation under relatively high light (incubation irradiance of 1,000 µmol quanta m^{-2} s^{-1}), the cells in these samples were more efficient at keeping PSII oxidized during exposure to light compared to initial measurements when cells were acclimated to deep mixing (Fig. 5a). In contrast, cells incubated under lower irradiance (i.e., maximum midday intensity of 100 µmol quanta m^{-2} s^{-1} in the LL treatment) showed a decreased capacity to cope with light and use it to drive photochemistry on the second day of incubation. In these samples a larger number of PSII reaction centers became reduced (i.e., Φ_{PSII} decreased, Fig. 5b) following exposure to the actinic light, suggesting that the cells were less efficient at coping with exposure to light [e.g., by drawing electrons away from PSII, possibly due to a lack of light-inducible photoprotective strategies (Cardol et al. 2008)] compared to the first day of incubation.

This response demonstrates that photoacclimation occurs rapidly at the onset of stratification, and likely influences which taxonomic groups become dominant during a bloom. For example, photoacclimation typically leads to an increase in photosynthetic pigment content under decreased irradiance, such that more light is harvested by the photosynthetic apparatus. For both picoeukaryotes and *Synechococcus* populations in the LL nutrient enrichment experiment, similar cellular fluorescence increases were observed in all treatments and the control (relative to initial levels, Fig. 9). The incubation of these cells under low light intensities was therefore either associated with a concurrent cellular synthesis of pigments, or a community shift in which cells with higher pigment content became more abundant, replacing their low-pigment counterparts.

When nutrient limitation was reversed but the incubation irradiance was similar to levels that would be experienced at greater depths in the euphotic zone, i.e., in the LL nutrient enrichment experiment, nutrient additions had a smaller effect on photosynthetic biomass than in the HL nutrient enrichment experiment. Specifically, the increase in Chl *a* (Fig. 6b) observed under low light conditions did not correspond to increased photosynthetic biomass in the LL nutrient enrichment experiment (Fig. 8b) even though shifts in the phytoplankton community were observed relative to initial samples (Fig. 7b). For example, picoeukaryote cell concentrations in the LL nutrient enrichment experiment increased over an order of magnitude regardless of nutrient addition treatment (Fig. 7b.1). (Picoeukaryote cell numbers in the deep euphotic zone during the spring bloom show similar trends, increasing at 120 m (Fig. 1e)). In contrast to the picoeukaryotes, *Synechococcus* and *Prochlorococcus* declined slightly in abundance for all treatments and the control in the LL nutrient enrichment experiment (Fig. 7b.2, b.3), possibly due to the presence of small (<20 µm) grazers that were able to pass through the mesh at the start of the experiment (Sommer 2000). The results of this LL nutrient enrichment experiment show that when light (rather then nutrients) limits growth, both photoacclimation and considerable shifts in community composition can occur within the phytoplankton community without a net gain or loss of photosynthetic biomass. Therefore, the traditional interpretation of "bloom" dynamics (i.e., as an increase in photosynthetic biomass) may be confined to the upper euphotic zone where light is not limiting, while other acclimation processes are more ecologically relevant at depth.

While *Synechococcus* declined in abundance during the LL nutrient enrichment experiment, samples from the deep euphotic zone during the spring bloom show a slight increase in *Synechococcus* abundance at 100 m (Fig. 1d) along with a concurrent increase in cellular fluorescence (Fig. 2), suggesting that *Synechococcus* cells are able to grow and acclimate within the light limited regions of the deep euphotic zone. Low-light adapted *Prochlorococcus* ecotypes have also been shown to be dominant and ubiquitous members of deep euphotic zone phytoplankton communities (West and Scanlan 1999; Rocap et al. 2003; Mackey et al. 2008) but did not grow in the LL experiments. One explanation for the difference in *Synechococcus*

and *Prochlorococcus* growth responses between cells in the LL nutrient enrichment experiment and cells from the deep euphotic zone during the spring bloom could be the different antecedent conditions to which these populations were acclimated prior to sampling. The LL nutrient enrichment experiment was conducted with water and phytoplankton collected from stratified surface waters; therefore, it is likely that these cells were acclimated to high light and may have required more than 4 days to adjust to lower incubation irradiances before growing. Moreover, low-light adapted ecotypes were likely less abundant in the sample water used in the nutrient addition experiments than high-light ecotypes (Fuller et al. 2005; Lindell et al. 2005), and would have required more time to become numerically dominant following a light shift. In contrast, cells sampled from the deeply mixed water column (>300 m) would likely be acclimated to a very different light regime, as before the bloom the water column was mixed from the surface (high light) to below the euphotic depth (very low light). Following stratification of the water column, cells trapped at depth would be primed to acclimate to low irradiances, allowing them to bloom immediately (Fig. 1d, e). In addition, larger seed populations of competent low light adapted ecotypes would have been present in the mixed water column than in the surface waters used during the LL nutrient enrichment experiment.

Our data suggest that in the Gulf of Aqaba, physiological acclimation to different nutrient and light regimes help shape the phytoplankton community. However, it is important to note that this physiological flexibility is likely only one factor influencing phytoplankton survival and community succession: multiple taxonomic groups of picoeukaryotes (Moon-van der Staay et al. 2001; Worden 2006), *Synechococcus* (Fuller et al. 2005; Penno et al. 2006), and *Prochlorococcus* (Moore et al. 1998; Rocap et al. 2003) coexist in this and other open ocean regions, and this extra layer of diversity may further enable picophytoplankton communities to adjust rapidly to changes in nutrient and light availability. The dominance of one group of picoeukaryotes during a bloom may therefore be at least partially attributable to the ability of different ecotypes within that group to thrive under different environmental nutrient and light regimes, rather than entirely through sustained expression of nutrient and light stress-response genes (Lindell et al. 2005).

Clarifying the combined influence of nutrient and light limitation on phytoplankton strategies and bloom dynamics in oligotrophic regions such as the Gulf of Aqaba is important for understanding how oceanic productivity could be affected by global change. Estimates suggest that by the year 2050 climate warming will lead to longer periods of stratification with fewer deep mixing events in seasonally stratified seas, causing expansion of low productivity, permanently stratified subtropical gyre biomes (i.e., ultraoligotrophic waters) by 4.0% in the Northern Hemisphere and 9.4% in the Southern Hemisphere (Sarmiento et al. 2004). Following oligotrophic ocean expansion, the effect of non-deepwater nutrient sources (atmospheric dry deposition, precipitation, nitrogen fixation, etc.) on oceanic net carbon sequestration, export production, and climate could become more significant as the relative contribution of deepwater nutrients decreases. This shift in nutrient availability and source, which would be overlaid upon a more static light environment, could substantially alter phytoplankton community structure and bloom dynamics in these regions. Our results from the oligotrophic Gulf of Aqaba suggest that under present-day conditions the introduction of exogenous nutrients into stratified surface waters supports blooms of picoeukaryotes that, *when present*, increase in number quickly by exploiting the new nutrients (Figs. 7a.1, 8; Labiosa 2007). However, because survivalists like *Prochlorococcus* are more likely to be abundant following prolonged ultraoligotrophic periods (Lindell and Post 1995; this work), the effect of exogenous nutrient sources (e.g., dust, nitrogen fixation, precipitation, etc.) on carbon sequestration within future permanently stratified oligotrophic seas remains difficult to predict. Specific investigations on the effects of exogenous new nutrients are still needed to fully understand if and how they will influence primary production and shape phytoplankton community structure in the oligotrophic ocean at present and in the future.

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